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THE RED TURNIP BEETLE, ENTOMOSCELIS AMERICANA BROWN (COLEOPTERA: CHRYSOMELIDAE), BIOLOGY AND PLANT RELATIONSHIPS

BY

© D. BRUCE STEWART

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Red Turnip Beetle, Entomoscelis americana Brown (Coleoptera: Chrysomelidae), Biology and Plant Relationships" submitted by Donald Bruce Stewart in partial fulfilment of the requirements for the degree of Master of Science.

Brian Hocking
Supervisor

K. Ghani

W. G. Evans

Dated 5 July 1973

ABSTRACT

The life history of *Entomoscelis americana* Brown was followed for the 2 years of the study, 1971 and 1972. Distribution in North America includes the 4 western Canadian provinces, Yukon Territory, Alaska, Washington, Idaho, Montana, Wyoming, Colorado, North Dakota, Wisconsin, and Minnesota. The range of *E. americana* is limited by mean daily July temperatures in excess of 70° F and below 55° F, and by annual precipitation in excess of 32 inches. No evidence of a lower limit of precipitation was found. Diapause was terminated in the laboratory by cold treatment. The larvae and newly-emerged adults are photo-positive. Aestivating adults are photo-negative and show thigmokinesis in conjuction with moist substrate. Following aestivation, the adults are again photo-positive. Dispersal occurs in August, following which, males respond to female odours. *E. americana* is oligophagous and restricted to the Cruciferae. Food plant finding by the larvae is largely by trial and error, although food plant odours act as arrestants at close range and non-food plant odours can act as repellents. Adults respond to food plant odours during August dispersal. Food plant acceptance is determined by at least 2 plant chemicals in the Crucifereae: an aldehyde stimulates larval feeding and synergises with a compound that has phenolic properties to stimulate adult feeding.

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W. F. Barr, Department of Entomology, University of Idaho, Moscow, Idaho.

W. J. Brandvik, North Dakota Department of Agriculture, Bismark, N.D.

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TABLE OF CONTENTS

1. INTRODUCTION 1

2. LITERATURE REVIEW 3

2.A. *Entomoscelis americana* Brown 3

2.B. Plant selection by phytophagous insects 4

3. ENTOMOSCELIS AMERICANA BROWN 12

3.A. Geographical distribution 12

3.B. Description of stages 16

3.B.i. *Egg* 16

3.B.ii. *larvae* 16

First instar - 16

Second instar - 18

Third instar - 18

Fourth instar - 18

Fifth instar - 19

3.B.iii. *Pupa* 19

3.B.iv. *Adult* 19

3.C. Life Cycle and habits 22

3.C.i. *Hatching* 22

3.C.ii. *Habits of the larvae* 22

3.C.iii. *Habits of the adults* 22

3.D. Laboratory rearing of *E. americana* 24

3.D.i. *Rearing techniques* 24

3.D.ii. *Termination of diapause* 25

3.E. Adult taxes and kineses 27
3.E.i. *Materials and methods* 27
3.E.ii. *Results* 29
3.F. Olfactory response of males to females 29
3.F.i. *Materials and methods* 32
3.F.ii. *Results* 34
4. FOOD PLANT RELATIONSHIPS OF E. AMERICANA ... 36
4.A. Food plant finding by the larvae 36
4.A.i. *Materials and methods* 37
4.A.ii. *Results* 38
4.B. Food plant finding by the adults 42
4.B.i. *Materials and methods* 42
4.B.ii. *Results* 43
4.C. Food plant acceptance 45
4.C.i. *Materials and methods* 45
4.C.ii. *Results* 52
5. CONCLUSIONS AND DISCUSSION 59
6. REFERENCES 63
7. APPENDICES 73
I. The photo-response of adults of *Entomoscelis americana*..... 73
II. The thigmokinetic response of adults of *Entomoscelis americana*..... 75

Appendix III. The response of males of *Entomoscelis americana* to the odour of gravid females..... 76

IV. The response of first instar larvae of *Entomoscelis americana* to the odour of rape foliage in 2-way choice chambers.... 77

V. The response of first instar larvae of *Entomoscelis americana* to the odour of food plants and non-food plants in a moving air olfactometer..... 78

VI. The response of adults of *Entomoscelis americana* to odours of rape foliage and blossoms..... 81

VII. The response of *Entomoscelis americana* to lettuce leaf discs treated with chemical fractions of rape foliage..... 83

LIST OF TABLES

Table 1. The dimensions of all stages of *Entomoscelis americana* 21

Table 2. The percent hatch of eggs of *Entomoscelis americana* following cold treatment 26

Table 3. The photo-response of adults of *Entomoscelis americana* in a light-dark choice chamber 31

Table 4. The thigmokinetic response of adults of *Entomoscelis americana* in a moist-dry choice chamber 33

Table 5. The response of males to the odour of gravid females in a moving air olfactometer 35

Table 6. The response of first instar larvae of *Entomoscelis americana* to the odours of food plants and non-food plants in a moving air olfactometer 40

Table 7. The response of first instar larvae of *Entomoscelis americana* to growing food plants and non-food plants in a choice chamber 41

Table 8. The response of adults of *Entomoscelis americana* to odours of rape foliage and blossoms in a double air-stream olfactometer 44

Table 9. The non-cruciferous plants tested for acceptability with *Entomoscelis americana* 49

Table 10. The response of *Entomoscelis americana* to lettuce leaf discs treated with chemical fractions of rape foliage 54

LIST OF FIGURES

Fig. 1. The distribution of *Entomoscelis americana* in North America 15

Fig. 2. The choice chamber used to evaluate moisture and thigmokinetic responses of *Entomoscelis americana* 30

Fig. 3. The moving air olfactometer used to evaluate olfactory responses in *Entomoscelis americana* 30

Fig. 4. The 2-way, food choice chamber used to evaluate the response of *Entomoscelis americana* to food plants, non-food plants, and chemical extracts of rape seed foliage 47

Fig. 5. A flow diagram outlining the procedure used to refine a chemical feeding stimulant from rape seed foliage 57

INTRODUCTION

The red turnip beetle, *Entomoscelis americana* Brown was first collected in Canada in 1825 during Sir John Franklin's second northern land expedition, and until 1941 was considered identical to the Eurasian species, *Entomoscelis adonidis* Pallas. The North American species was re-described as *E. americana* in 1941 by Brown (1942).

Both larval and adult stages of *E. americana* are oligophagous and restricted to cruciferous plants. The adults were first reported attacking cultivated plants at Regina in 1885, and minor outbreaks occurred on the Canadian prairies in 1918, 1927, and 1936. The red turnip beetle is a sporadic pest of cruciferous garden crops in the interior of Alaska, and along the Mackenzie River valley in the North West Territories.

During the past 30 years, *E. americana* received little attention in Canada since it is not a pest of the cereal and forage crops that have been prominent in western Canadian agriculture. It has been largely ignored by American workers as its range in agricultural areas is chiefly restricted to the Canadian North-West.

The preference of *E. americana* for cruciferous plants causes it to be an intermittent, but often severe pest of the increasing rape acreage (*Brassica campestris* L.) in Western Canada. Although the beetle completes its larval stages before most rape crops are sown, the adults emerge when these crops are in the seedling stage, and it is at this time, especially in the parkland areas of the

prairies provinces; that the red turnip beetle is often a severe agricultural pest.

The object of this study is twofold: firstly, to provide a comprehensive description of the stages, life cycle and habits of *E. americana*, and secondly, to investigate the mechanism of food plant selection.

Field studies were conducted in the St. Albert area of Alberta during the growing seasons of 1971 and 1972. Experimental work pertaining to food plant selection, taxes and kineses, embryological development, and the development of rearing techniques were carried out in the laboratories of the Department of Entomology, University of Alberta. The experimental plants used in this study were cultured in the greenhouses of the Plant Science Department, University of Alberta, on the university campus.

2. LITERATURE REVIEW

2.A. *Entomoscelis americana* Brown

The Eurasian red turnip beetle was first noted in the literature by Pallas (1771) as *Chrysomela adonidis*, the specific name derived from the food plant *Adonis autumnalis* L., a herbaceous annual of the family Ranunculacea. The same insect species was described by J. C. Fabricius in 1777 (Zimsen, 1964) as *Chrysomela trilineata*, and designated *Entomoscelis adonidis* by Redtenbacher (1874). Under this latter designation, adult morphology was described by Linné (1789) and larval morphology by Weise (1893).

The first report of the North American red turnip beetle was from Manitoba, near Latitude 54° N, in 1825; several specimens were collected during Sir John Franklin's second inland expedition, designated *adonidis*, and placed in the genus *Phaedon* Latreille by Kirby (Richardson, Swainson, and Kirby, 1837). Fletcher (1893) and Chittenden (1902) described the life history and both followed Kirby in referring North American specimens of *Entomoscelis* Chevrolat to the Eurasian *adonidis* Pallas. Powell (1941) also used this designation in his description of the genitalia. Because of structural differences between the Eurasian and North American species, Brown (1942) re-described the latter as *Entomoscelis americana*.

The first cultivated crop recorded attacked by *E. americana* was turnip at Regina in 1885 (Fletcher, 1888). The Canadian Insect Pest Review (1924) listed later reports and included the known distribution of the red turnip beetle in Canada. First reports of

attacks on cruciferous vegetable crops in other areas include Manitoba in 1890, Alberta in 1900, British Columbia in 1905, and the Yukon Territory in 1914. The red turnip beetle attracted attention in Western Canada during minor outbreaks at Edmonton in 1918, (Whitehouse, 1919), Saskatchewan in 1927 (King, 1928), and in the park belt and adjacent prairie regions of Saskatchewan and Alberta in 1936 (Twinn, 1936). Arnason (1948) and Strickland and Hocking (1950) outlined control methods, and a brief description of the red turnip beetle was provided by Beirne (1971).

2.B. Plant selection by phytophagous insects

Early workers recognized that many phytophagous insects are restricted to particular plant taxa for feeding or oviposition. Burmeister (1836) noted that certain flea beetles display a marked preference for cruciferous plants. Fabre (1918) referred to a similar preference in the cabbage butterfly, *Pieris rapae* L. as "botanical instinct", and wrote,

> "The botanist, to recognize a crucifer, requires the indication provided by a flower. Here *Pieris* surpasses us ... and she knows this group of plants to perfection." (p. 294, 295).

The classical work of Verschaeffelt (1910) removed part of the mystery of Fabre's botanical instinct by demonstrating that mustard oil glucosides, common in cruciferous plants, are responsible for attracting *Pieris rapae*. Brues (1920) suggested that such botanical instinct may be nothing more than extreme sensitivity to a complex of chemical and physical stimuli provided by the plants. Food plant

discrimination by lepidopterous larvae was shown by Dethier (1937) to involve both gustatory and olfactory stimuli.

The literature from 1864 to 1924, on plant specificity in phytophagous insects was reviewed by Brues (1924). Thorsteinson (1960) provided a review to 1959 and Jacobson (1966) continued the review to 1965.

Person (1931) proposed that volatile components in pine bark such as aldehydes and esters were responsible for attracting the western pine beetle, *Dendroctonus brevicomis* LeConte to its host, however he cited little evidence to support his proposal. As the result of a limited number of tests, Dethier (1941) reported that several essential oils of a number of umbelliferous plants were attractive to larvae of the swallowtail, *Papilio asterus* Cramer.

During later years many botanical compounds, of little importance to insects nutritionally, were reported attractive to various, specialized phytophagous insects. In well-documented studies, Thorsteinson (1953) and Nayar and Thorsteinson (1963) showed that the larvae of the diamondback moth, *Plutella maculipennis* Curtis were stimulated to feed by several mustard oil glucosides extracted from tissues of plant species in the families Crucifereae and Tropaeolaceae. Alcohols and aldehydes in mulberry leaves were reported attractive to larvae of the silkworm, *Bombyx mori* L. (Watanabe, 1958; Hamamura, 1959; Nayar and Fraenkel, 1962). By means of a simple bio-assay method, employing plant extracts on filter paper, Yamamoto and Fraenkel (1959) showed that unidentified, botanical glucosides were responsible for food plant selection by

larvae of the tobacco hornworm, *Protoparce sexta* Johanson, and the Colorado potato beetle, *Leptinotarsa decemlineata* Say. Coumarin, an odorous principle of sweet clover plants, attracted the sweet clover weevil, *Sitona cylindricollis* Fahraeus (Thorsteinson, 1960). Nayar and Fraenkel (1963) provided strong evidence to show that glucosides from the catalpa tree elicit a definite response from the catalpa sphinx, *Ceratomia catalpae* Boisduval.

Feeding stimulants for the boll weevil, *Anthonomus grandis* Boheman include volatile extracts from the leaves of cotton seedlings (Keller *et al.*, 1963; Neff and Vanderzant, 1963), and water extracts from all parts of the cotton plant (Jenkins *et al.*, 1963). Loschiavo, Beck, and Norris (1963) reported an unidentified feeding stimulant from the American elm attractive to the smaller European elm bark beetle, *Scolytus multistriatus* Marsham. This feeding stimulant was later identified as a pentacyclic triterpene (Baker and Norris, 1967) and represents the first chemical stimulant to be isolated from the woody tissue of a perennial plant. Derr, Randall, and Kieckhefer (1964) extracted an unidentified, non-nutritional component from corn plants that had a stimulatory effect on the corn rootworms, *Diabrotica longicornis* Say and *D. vergifera* LeConte. In a brief report that lacked supporting evidence, Chambliss and Jones (1966) reported that certain tetracyclic triterpenes, bitter principles present in the Cucurbitaceae, were attractive to the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber. In a well-documented study, David and Gardiner (1966) reported that 9 mustard oil glucosides extracted from the Crucifereae elicited a

strong response from the European cabbageworm *Pieris brassicae* L. As the result of an in-depth study involving many field experiments, Feeny, Paauwe, and Demong (1970) demonstrated that allyl isothiocyanate, an aglycone isolated from cruciferous plants, was a powerful attractant for adults of the flea beetle, *Phyllotreta cruciferae* Goeze.

Of the nutritional plant components that have a stimulatory effect, sucrose is one of the most widespread. This effect was demonstrated in the Mexican bean beetle, *Epilachna varivestis* Mulsant (Nayar and Fraenkel, 1963a; Augustine *et al.*, 1964), the European corn-borer, *Pyrausta nubilalis* Hubner (Beck, 1965), and the aphid *Myzus persicae* Sulzer (Dethier, 1966). Amino acids are generally non-phagostimulant, but the exceptions are of interest. Thorpe *et al.* (1947) presented considerable evidence to show that several amino acids caused aggregation in wireworms. In well-designed experiments employing agar-based media, Beck and Hanec (1958) demonstrated that larvae of *Pyrausta nubilalis* were stimulated to feed by several amino acids. A similar effect in the large milkweed bug, *Oncopeltus fasciatus* Dallas was reported by Feir and Beck (1963).

Amino acids also synergise with other compounds. Serine and glucose enhance feeding by larvae of *Pyrausta nubilalis* (Beck and Hanec, 1958); both leucine and methionine increase the acceptability of sucrose by *Myzus persicae* (Mittler and Dadd, 1964). Proline and glutamic acid synergise with sucrose for larvae of the spruce budworm, *Choristoneura fumiferana* Clements (Heron, 1965). Synergism reported among other compounds include sucrose and sinigrin for

larvae of *Plutella maculipennis* (Thorsteinson, 1953; Nayar and Thorsteinson, 1963), glucose and an unidentified plant component for *Protoparce sexta* (Yamamoto and Fraenkel, 1959), and glucose, glucosides, and an unknown volatile plant component for *Epilachna varivestis* (Nayar and Fraenkel, 1963a). As the result of an extensive behavioural, ultrastructural, and electrophysiological study, Chun, (1972) demonstrated that sucrose synergises with mustard oil glucosides, amino acids, salts, and ascorbic acid for larvae of *Pieris brassicae*.

The fact that both nutritional and non-nutritional plant chemicals have a stimulatory effect gave rise to considerable controversy regarding the mechanism of food plant selection in oligophagous, plant-eating insects. Two subordinate questions from which this controversy arose were cited by Dethier (1966):

> "Are the effective stimuli token or nutrient? Is oligophagy based on the unique acceptance of particular compounds, or rather, general rejection of many?" (p. 48).

Fraenkel (1953, 1959) contended that the leaves of all plants were nutritionally adequate for phytophagous insects, and that food plant specificity was based on the presence of secondary plant chemicals acting as token stimuli. He noted that many plants contained non-nutritional chemicals such as glucosides, alkaloids, and essential oils, and since these chemicals appeared to have no known role in basic plant metabolism, he referred to them as secondary chemicals. As the result of criticism of his generalization regarding food plant specificity (Thorsteinson, 1960; Waldbauer, 1962;

Beck, 1965; Kennedy, 1965), Fraenkel (1969) conceded that nutritional values may vary slightly in plants. He also admitted that certain plant nutrients may act as attractants, but maintained that this could not explain plant specificity in the specialized insects since plant nutrient composition varied greatly with season, soil, age, and light conditions, yet plant specificity was relatively unaffected by such variations.

Jermy (1966) contended that plants developed secondary chemicals as deterrents to herbivores, and insect sensitivity to such deterrents was more important in determining food plant range than adaptation to specific token stimuli. Kennedy (1958) favoured a "dual discrimination" theory of food plant selection involving response to both nutrient and token stimuli. Thorsteinson (1960) maintained that food plant finding was by chance, and plant acceptance initiated by secondary chemicals that act as arrestants and also lower response thresholds to nutrient phagostimulants that release feeding activity.

No single theory of food plant selection can be satisfactorily applied to all the specificities existing between phytophagous insects and plants. Fraenkel's (1959) token stimuli theory may explain plant selection in several lepidopteran species (Ehrlich and Raven, 1964), but does not explain the relationships between many aphids and their food plants where nutritional components play a significant role (Kennedy, 1958). Jermy's (1966) chemical deterrent theory may explain the comparative immunity enjoyed by certain plants, but does not explain food plant selection *per se*.

Kennedy's (1958) dual discrimination theory has the advantage of flexibility with regard to the role of physiological changes in the insect and its food plant, however it assumes that all phytophagous insects are capable of nutritional assessment of food plant value. Verification of Thorsteinson's (1960) theory that secondary chemicals lower response thresholds to sapid nutrients will require detailed electrophysiological analyses of receptor systems as suggested by Schoonhoven (1968) and then may only apply to certain plant-feeding insects.

Much of the controversy regarding the mechanisms of plant selection by oligophagous insects stems from the fact that most research into factors regulating quality discrimination emphasizes nutritional rather than ecological factors. From an ecological viewpoint, the plant may often be considered a place to live as well as a feeding substrate (Kennedy, 1953; Dethier, 1970). Plant selection may be a compromise between meeting the nutritional requirements of the insect, and such ecological considerations as habitat selection (Holloway, 1964), predators (Brower, 1958; Brower and Brower, 1964; Alpin, Benn, and Rothschild, 1968), and parasitization (Downey, 1962; Stride and Straatman, 1962).

Dethier (1954) contended that the diverse patterns of feeding habits in phytophagous insects represented a dynamic equilibrium between two evolving systems, the plants and the insects. The effects of such co-evolution on particular adaptations in plants and insects was discussed by Breedlove and Ehrlich (1968) and Muller (1969). Plants are evolving in an atmosphere of

multiple selective pressures of which insect predation is but one, and respond to these pressures by developing morphological modifications, emigration to new habitats, and the elaboration of new chemicals. Phytophagous insects are evolving new feeding habits as a result of structural modifications, metabolic modifications that overcome plant toxins such as glucosides, alkaloids, and tannins, and neural modifications that permit behavioural diversity (Dethier, 1970). The types of insect-plant relationships will vary as the evolutionary pathways which culminate in them vary. Consequently, no single theory of plant selection will apply to all the specificities existing between insects and plants, and many of these must be investigated separately.

3. ENTOMOSCELIS AMERICANA BROWN

3.A. Geographical distribution

Prior to 1942, the geographical distributions of *Entomoscelis americana* and *E. adonidis* were combined, as these species were then considered identical.

LeConte and Horne (1883) described the range as the boreal regions of North America and Eurasia. Hamilton (1894) reported the North American distribution as everywhere through the Rocky Mountains to 11 thousand feet, as well as Montana, the Hudson's Bay region, Alberta, Manitoba, and the North-West British provinces, and the Eurasian distribution as Southern Europe, France and Germany, and west and east Siberia to Turkestan. Chittenden (1902) reported that the range consisted of the boreal parts of both the Old and New Worlds. Leng (1920) gave the North American distribution as the Hudson's Bay Territory, Montana, Alberta, and Colorado. Essig (1926) listed the distribution as boreal North America, Siberia, and Europe, with the North America distribution including Colorado, Montana, Washington, British Columbia, and Alaska. Beller and Hatch (1932) reported that the Eurasian range extended from Southern France and Bavaria to Persia and Siberia, and showed the North American range as extending from Alaska and the Hudson's Bay region to Montana, Colorado, and Washington.

Figure 1 shows the known distribution of *E. americana* in North America, and was compiled from data received from United States Department of Agriculture, Economic Insect Survey Co-

ordinators, and from federal and provincial departments of agriculture in Canada.

The distribution of *E. americana* in British Columbia is confined to the central portion of the province and has not been reported from the coastal regions. In the southern, interior portion of British Columbia, distribution is restricted to higher elevations. Such distribution in British Columbia suggests a preference for a cool, fairly dry climate, so that the range of *E. americana* may be limited by high summer temperatures and high precipitation. Absence from areas of high summer temperatures is also evident in Washington, Idaho, Montana, Wyoming, and Colorado, where distribution is restricted to higher elevations. In the Dakotas, *E. americana* is only reported from the extreme northern portion of North Dakota, where the average daily July temperature does not exceed 70° F. *E. americana* has not been reported from Ontario, although its range does extend to Minnesota and Wisconsin where annual precipitation is comparable to that of Western Ontario. Although *E. americana* has not been reported from Western Ontario, it is probably present in this region, especially in settlements where market gardens are present.

Figure 1 shows the known distribution of *E. americana* in relation to mean daily July temperature and to average annual precipitation. The July isotherm was chosen since, in Alberta, *E. americana* spends this month in aestivation. The southern limit of *E. americana* does not extend below the 70° F July isotherm, suggesting that its range is restricted by high summer temperature.

The limit of northern distribution coincides with the July 55° F isotherm which also lies fairly close to the permafrost line in Northern Canada. The known range of *E. americana* also appears limited by annual precipitation in excess of about 32 inches, which may explain its apparent absence from Eastern Canada and the coastal regions of British Columbia.

The 70° F July isotherm and the isohyets plotted on Figure 1 are from Chapman and Sherman (1967). The Alaskan 55° F July isotherm is from Watson (1959), and the Canadian 55° F July isotherm is from Thomas (1953).

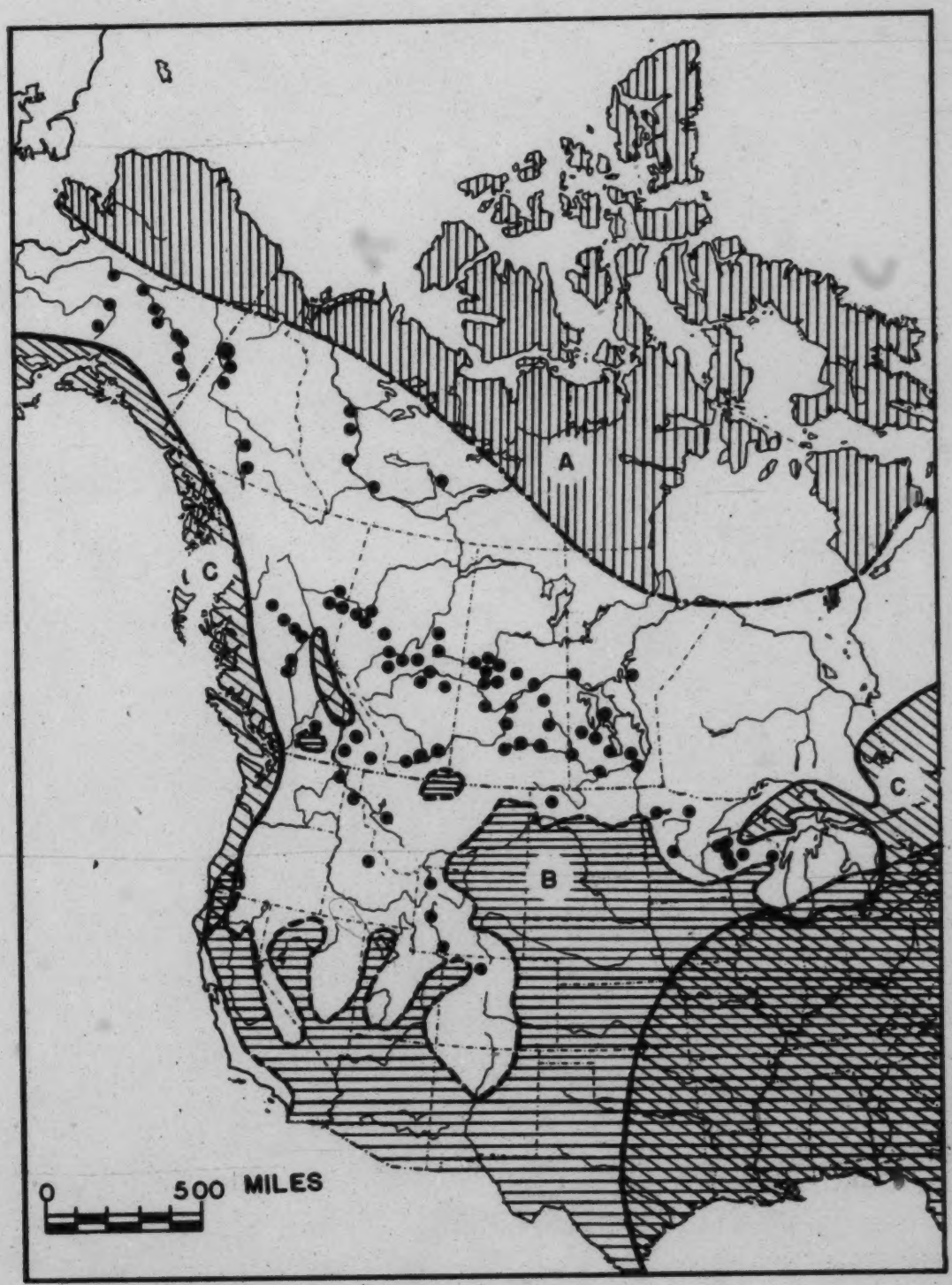

Fig. 1. The distribution of *Entomoscelis americana* Brown in North America in relation to temperature and precipitation: A, mean daily July temperature below 55° F; B, mean daily July temperature above 70° F; C, annual precipation in excess of 32 inches.

3.B. Description of stages

3.B.i. *Egg*

When first laid, the eggs are bright orange-red, gradually darkening to dark brown. The egg surface is finely reticulated with small hexagonal areas of about 0.15 mm diameter. The egg shape is a prolate spheroid with the length about twice the diameter. Length and diameter measurements are of the order reported by Chittenden (1902), 1.5 X 0.7 mm (Table 1).

3.B.ii. *Larvae*

There are 5 larval instars, the average measurements of which are shown in Table 1. Larval length measurements vary because of the extendable abdomen and, accordingly, were taken from specimens preserved in 70% ethanol. Head capsule widths were also recorded. The average ratio of increase of head capsule width in successive instars is approximately 1.34.

First Instar - The newly-hatched larvae are orange-yellow, except for the mouthparts, antennae, stemmata, thoracic legs, and a pair of laterally-placed tubercles on each thoracic segment, which are all black. The dorsal surface darkens to near black within 6 hours of hatching; the ventral surface also darkens but retains a slight orange hue. The first instar larva is wedge-shaped, about 2.0 mm in length by about 0.75 mm in width at the head capsule which is the widest part.

The mouthparts are of the generalised, chewing type. A wide, curved clypeus largely overlies a narrower, bi-lobed labrum. Each

of these structures bears 4 large bristles. The mandibles are wide between the condyle and ginglymus, and each bears a well-developed retinaculum as well as 2 large bristles. The laciniae are short and have cuticular spines at their apices. The maxillary palpi are dome-shaped, 4-segmented, and each has 14 basiconic sensilla at the apex. The labium is short and narrow and is equipped with 6 short bristles at the distal end and 2 longer bristles at the posterior end. The labial palpi are 2-segmented and each bears 12 basiconic sensilla at the apex. The antennae are wide-set and each consists of a single article or scape set in a well-defined antennal socket. Each antenna has 4 trichoid sensilla at the distal end. The stemmata are laterally-placed and located immediately behind the antennal sockets. There are 6 stemmata on each side of the head capsule, 4 of which are arranged in a square and lie slightly above a subordinate pair. The thoracic legs are all 3-segmented, each bearing numerous hairs and terminating in a single, simple claw. Each thoracic segment bears a pair of dorsal, transversal rows of fuscous tubercles, with from 6 to 8 tubercles in each row. Also located on each thoracic segment is a pair of laterally-placed, prominent tubercles just above the stigmatal line. Smaller tubercles are also located laterally on each thoracic segment below the stigmatal line, and 2 smaller series on the ventral surface. There are 7 abdominal segments. The first 4 have a pattern of tubercles similar to the thoracic segments, except that the prominent, lateral tubercles are lacking. The 5th and 6th abdominal segments taper abruptly to terminate in a bi-lobed, prehensile structure used in locomotion.

Second Instar - After the first moult, the body remains wedge-shaped, with an average length of about 3.3 mm. The width of the head capsule is about 0.98 mm. The body is flattened on the ventral surface and rounded above. The head is sub-rotund, slightly depressed at the apex, and abruptly truncate at the anterior end. The appendages, mouthparts, and stemmata are the same as in the previous stage. The dorsal surfaces of the abdominal and thoracic segments are divided by transverse folds. The ecdysial suture is apparent as a narrow, slightly-depressed, dorsal groove running from the apex of the head to the posterior abdominal segments, and is more distinct where it crosses the thoracic tergites. The colour of the body is velvet-black on the dorsal surface and blackish-orange on the ventral surface. The tubercle pattern is the same as in the previous stage.

Third Instar - After the 2nd moult, the body is less wedge-shaped and more elongate. The head capsule and thoracic segments are approximately the same width - about 1.28 mm, and the body length is about 5.2 mm. Tubercle pattern, colour, and appendages are the same as in the previous stage.

Fourth Instar - The body length is about 8.1 mm, and the head capsule width about 1.67 mm. The head capsule is slightly narrower than the thoracic segments, providing the body with a more elongate shape. The ornamentation is the same as in the previous stage, except that the cuticle on the ventral surface is more translucent, providing a dull, orange hue in this region. A pair of small, indistinct projections is present on the ventral surface of

the 4th, 5th, and 6th abdominal segments.

Fifth Instar - The length of the final instar is about 12.0 mm, and the width of the head capsule about 2.17 mm. The body is more elongate in this stage, with the head capsule noticeably narrower than the thorax. The small projections on the 4th, 5th, and 6th abdominal segments are more pronounced in this stage and appear as 3 pairs of translucent, bag-like pseudopodia that can be extended from median slits in the ventral surface. These serve as prolegs and are used in conjunction with the bi-lobed, terminal abdominal segment during locomotion. The remaining ornamentation and colour is the same as in the previous stage, except that the ventral surface is yellow in many specimens.

3.B.iii. *Pupa*

The average pupal dimensions are 6.1 X 3.1 mm. The colour is chiefly bright orange, with the antennae, elytra, and leg cases yellowish-orange. The spiracles are rotund and fuscous. The elytra cases each bear 3 longtitudinal striae. The ecdysial suture is a shallow, median groove on the dorsal surface of the metathorax. The thorax is covered with fine, short bristles and similar bristles are arranged as median, transverse ridges on each abdominal segment.

3.B.iv. *Adult*

The form and colour pattern were described by Chittenden (1902), Fletcher (1905), and Brown (1942). The outline of the body,

from the dorsal aspect, is elongate oval. Average female body dimensions are 9.1 X 4.9 mm, while the males are smaller at 7.6 X 4.2 mm (Table 1).

The colour pattern is the same in both sexes. The dorsal surface of the head is red, and bears a small, black triangle on the median line at the Posterior margin. The mouthparts, clypeus, antennae, and small areas surrounding the eyes are black. The pronotum is red with a broad, black median area as well as a small spot on each side also in black. The black median area on the pronotum is bell-shaped, widest at the posterior edge, and covers about two-fifths of the tergite. The scutellum is a small, black triangle. The elytra are red, each with a narrow, black, sutural margin except near the scutellum, and a wider sub-median vitta that is pointed at each end and approaches, but does not meet, the base and apex of each elytron. The punctures of the pronotum, elytra, and sterna are fine, dense, and scattered.

The tarsal segments of the anterior and middle legs are wider in the male than in the female, and are probably of advantage to the males during copulation. The terminal, abdominal segments are flattened at the middle. The posterior margin of the abdomen is bisinuate in the male, and simply truncate in the female.

Table 1. The dimensions in mm of all stages of *Entomoscelis americana* Brown. Egg and larval measurements based on 10 observations, pupal and adult measurements based on 20 observations.

Stage	Length†	Width†
Egg	1.5 ± 0.11	0.7 ± 0.12
	(1.3 - 1.7)	(0.6 - 0.9)
First instar larva	2.1 ± 0.12	0.75 ± 0.013*
	(1.9 - 2.2)	(0.73 - 0.76)
Second instar larva	3.3 ± 0.10	0.98 ± 0.023*
	(3.2 - 3.4)	(0.96 - 1.01)
Third instar larva	5.2 ± 0.07	1.28 ± 0.043*
	(4.8 - 5.3)	(1.22 - 1.31)
Fourth instar larva	8.1 ± 0.43	1.67 ± 0.056*
	(7.6 - 8.7)	(1.59 - 1.73)
Fifth instar larva	12.0 ± 0.73	2.17 ± 0.059*
	(11.1 - 12.9)	(2.09 - 2.25)
Pupa	6.1 ± 0.91	3.1 ± 0.45
	(5.2 - 7.4)	(2.3 - 3.7)
Female Adult	9.1 ± 0.67**	4.9 ± 0.30
	(7.8 - 9.7)	(4.3 - 5.4)
Male adult	7.6 ± 0.49**	4.2 ± 0.23
	(6.8 - 8.2)	(3.7 - 4.5)

*head capsule width
**excluding antennae

†mean ±S.D.
(range)

3.C. Life cycle and habits

3.C.i. *Hatching*

E. americana is uni-voltine and overwinters as eggs in the soil. In 1972, eggs in the Edmonton area hatched during the first week of May. Muscular contractions in the embryo begin within 6 hours before hatching and are readily observed through the egg chorion. Eclosion is accomplished by means of a rupture of the vitelline membrane and chorion that originates anteriorly and then extends along the dorsal side of the egg. No cuticular hatching spines are evident on newly-hatched larvae.

3.C.ii. *Habits of the larvae*

The first instar larvae are stronly photo-positive and climb up plant stems soon after hatching. While no cultivated cruciferous crops are available in early May, the larvae feed on such cruciferous weeds as volunteer rape, *Brassica campestris* and shepherd's purse, *Capsella bursa-pastoris* (L.) Medic. The larvae feed both during the day and night. When disturbed, they drop to the soil, where their coloration blends well with that of the soil. The 5 larval instars are completed in about 3 weeks. The mature larvae then enter the soil to a depth of about 1 inch and form small, smooth cavities within which they pupate. The pupal stage lasts from 2 to 3 weeks.

3.C.iii. *Habits of the adults*

Most field studies were conducted on 2 adjoining, one-

quarter section fields in the St. Albert area, located on the North $\frac{1}{2}$, Section 12, Township 53, Range 35, West of the 4th Meridian. One field contained roughly one acre of volunteer rape at one corner which was left intact during the 2 years of the study. In 1972, the adults began emerging in the volunteer rape during the first week of June and populations were highest during the second week of June. Feeding activity is greatest during the first 3 weeks after emergence, and it is at this time that *E. americana* can cause considerable damage to cultivated rape crops.

At the beginning of July, *E. americana* begins a period of aestivation that lasts for about one month. During this period, the beetles are found under plant debris, in soil crevices, or buried in loose soil to a depth of about one inch. When the aestivation period ends, the beetles disperse by walking and flying, and are then found in previously uninfested rape fields or on patches of cruciferous weeds. The plants that served as food prior to aestivation are producing seed and have lost most of their leaves.

The 2 fields where much of this study was conducted were both sown in barley during the spring of 1972, and most of the weeds later destroyed with a herbicide. Many small, isolated patches of volunteer rape persisted in both fields however, these having sprouted from seed in late May. Such later growth has an abundance of blossoms and leaves, and appears to be an attractive source of food for the dispersing beetles. The isolated growths of volunteer rape in both

fields were checked twice-weekly during August and the beetles were found progressively further into the barley fields at each check. Three weeks after dispersal began, both fields were infested throughout, and at the end of August the beetles were discovered in a previously uninfested rape field one mile distant.

Immediately after dispersal, copulation occurs. Oviposition begins during the first week of August and continues for about one month. The eggs are deposited in masses of up to 80 on the soil beneath food plants and are found under leaf litter, in soil crevices, or buried in loose soil to a depth of about one-quarter inch. The abdomens of the females remain swollen after oviposition and these when dissected, are found to contain no eggs but rather, a yellow haemolymph. In the field, many specimens of *E. americana* die shortly after oviposition, while others persist until mid-October.

3.D. Laboratory rearing of *E. americana*

3.D.i. *Rearing techniques*

Larvae and adults were reared both directly on cabbage leaves and on potted rape plants in rearing cages. Openings in the floor of the rearing cage permitted the use of plastic planters that were suspended in trays of water beneath the cage. This system simplified watering of food plants and also provided that the soil in the planters and the floor of the rearing cage were at the same level, allowing easy access to food plants by the larvae.

Detached rape leaves are unsuitable as food, since such leaves, when detached for 3 days, are toxic to the larvae. A

naturally-occurring enzyme, such as a glucosidase, may be present in rape foliage. Although mustard oil glucosides are non-toxic, cleavage of such glucoside molecules will yield glucose and mustard oils. Many mustard oils, according to Ettlinger and Kjaer (1968) are strongly irritant and can cause damage to animal tissue.

Egg-gathering was simplified by maintaining copulating couples in small containers that were lined at the bottom with layers of paper towelling. The females oviposited between these layers, and the egg masses were easily brushed off when the paper towelling was changed.

3.D.ii. *Termination of diapause*

Daily, microscopic examination of freshly-laid eggs revealed that embryological development progresses for about 3 weeks. The late embryos then enter diapause which lasts, in the field, until the first week of May. Several thousand eggs that had completed embryological development were stored in a cold room at 15° C. After one week, all but 500 eggs were transferred to a cold room at 10° C, and one week later, all but 500 of these transferred to a cold room at 5° C. In this fashion, the eggs were successively introduced to lower temperatures until a minimum of -15° C was reached. Each week, 50 eggs were removed from each cold storage unit and returned to room temperature. These were placed on moistened discs of paper towelling in covered, Pyrex glass petri dishes and checked daily to determine the percentage hatch.

Table 2. The percent hatch of eggs of *Entomoscelis americana* Brown following temperature treatment to terminate diapause.

Temperature	Duration of treatment in weeks									
(°C)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
20	-	-	-	-	-	-	-	-	-	-
15	-	-	2	-	-	1	-	1	1	1
10	-	1	-	4	3	5	6	8	3	4
5	-	-	-	4	2	6	12	14	16	12
0	-	4	14	60	84	90	87	90	84	86
- 5	-	-	-	14	16	10	20	18	14	17
-10	-	-	-	-	-	1	-	-	2	-
-15	-	-	-	2	-	-	1	-	-	-

Table 2 shows the results of the cold treatment. Diapause was most effectively terminated by cold treatment at 0^{o} C, with maximum hatch occurring after 6 weeks exposure to that temperature.

Most of the eggs hatched between 24 and 36 hours after removal from the cold storage unit.

3.E. Adult taxes and kineses

While the photo-positive response of the larvae is obvious and requires no experimental confirmation, a similar, but discontinuous response that varied with time was observed in the adults. Experiments were designed to evaluate the photo-response of newly-emerged and aestivating adults. Other experiments were conducted to test the response of aestivating adults to moist substrate and also to determine if they showed thigmokinesis.

3.E.i. *Materials and methods*

The response of field-collected, newly-emerged adults to light was investigated by means of an opaque, plastic, cylindrical choice chamber (30.0 X 4.0 cm) that admitted light at one end only. The lighted end of the choice chamber was covered with a 5.0 cm, Pyrex glass petri dish and illuminated from above by a fluorescent light fixture equipped with a single, 15-watt daylight type, 16-inch tube. The light source was suspended 6 inches above the choice chamber and provided approximately 260 foot-candles of incident light at the lighted end of the chamber. Five groups of 20 adults were placed in the choice chamber for 2-hour

periods and the numbers in the lighted and darkened regions recorded at the conclusion of each trial. The intensity of reaction was determined from the ratio of the percentage in the lighted end to the percentage in the darkened end (L/D). Photo-responses were similarly investigated at the beginning, during, and at the end of the aestivation period.

The response of aestivating adults to moist substrate was determined by placing groups of 20 in a rectangular choice chamber (17.0 X 9.0 cm) that was lined at the bottom with a single layer of paper towelling. Five groups were placed in choice chambers with dry substrate, their positions recorded after 30 minutes, and then placed in choice chambers with one-half the paper substrate moistened, for a further 30 minutes. During these tests, the choice chambers were kept in a darkened container that was rotated 90° between trials to discount the effects of any stray light entering the system.

A thigmokinetic response in conjunction with moist substrate was investigated in aestivating adults by repeating the moist substrate tests with one-half the paper towelling folded to provide parallel, tunnel-shaped openings between the paper towelling and the floor of the test chamber (Figure 2). Five groups of 20 adults were placed in choice chambers with dry substrate for 30-minute periods, their positions recorded, and then placed in test chambers with the folded substrate moistened, for further 30-minute periods.

3.E.ii. *Results*

Table 3 shows the results of the tests with the light-dark choice chamber. The recordings are totals of 5 trials listed in Appendix I. Of the newly-emerged adults, a total of 94 were found at the lighted end of the chamber. At the beginning of the aestivation period, only 10 were found at the lighted end, and the response was roughly neutral when the aestivation period was about 75% complete. At the end of aestivation, a total of 89 were found at the lighted end.

In trials employing moist substrate, all the aestivating adults responded positively to the moistened paper towelling, while in trials employing dry substrate the beetles were located randomly in the chamber.

The results of the tests to determine thigmokinetic response to openings in moist substrate are shown in Table 4 which lists the totals of 5 trials listed in Appendix II. In trials employing dry substrate, the beetles were dispersed randomly in the test chamber and a total of 9 were found in the openings provided by the folded paper substrate. When the folded paper substrate was moistened all the beetles were found at the moist end of the test chamber and a total of 77 were found within the openings.

3.F. Olfactory response of males to females

Approximately one week after completion of the aestivation period, copulation begins. Experiments were conducted in the laboratory to determine if the males, at this time, would respond to the

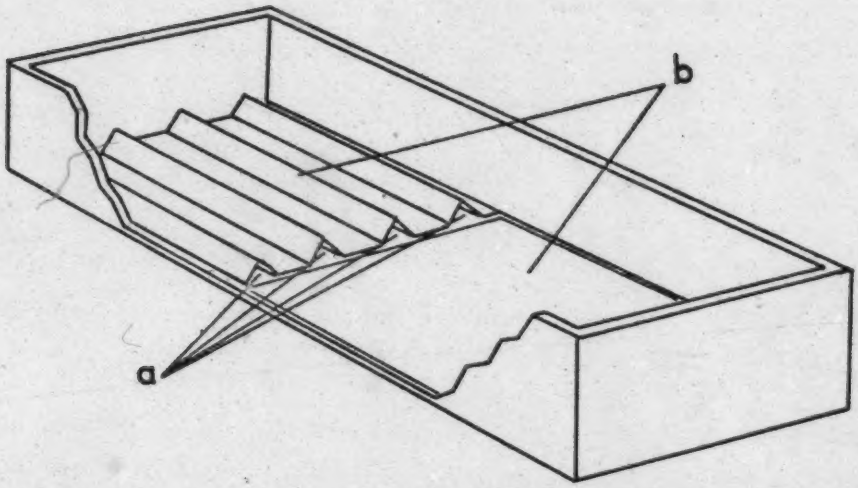

Fig. 2. The choice chamber used to evaluate moisture and thigmokinetic responses of aestivating adults of *Entomoscelis americana* Brown: a, tunnel-shaped openings between paper towelling and the floor of the test chamber; b, paper towelling.

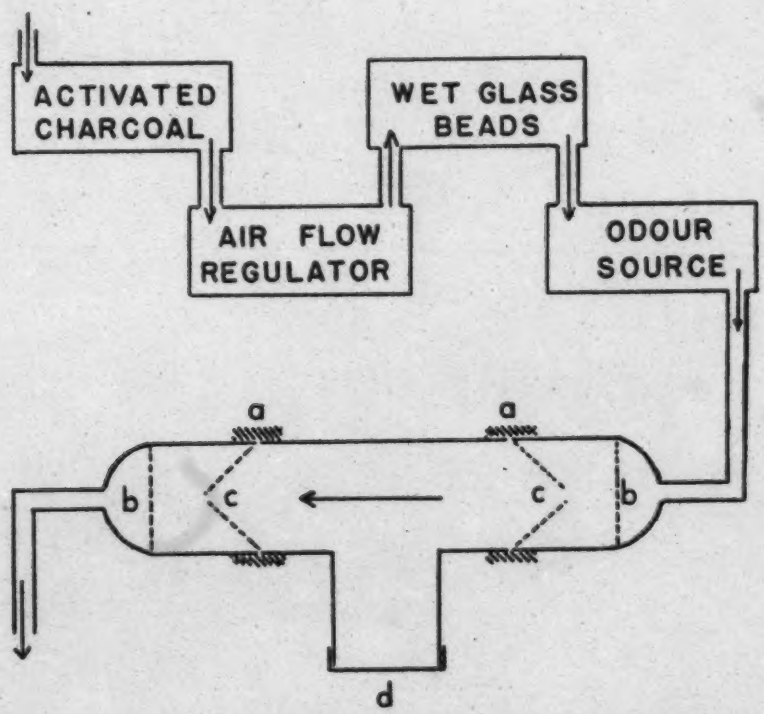

Fig. 3. A T-tube apparatus used to evaluate olfactory responses of *Entomoscelis americana* Brown: a, plastic tube couplings; b, flat 20-mesh, saran screen discs; c, 75°, 20-mesh saran screen cones; d, opaque plastic cover.

Table 3. The photo-response of adults of *Entomoscelis americana* Brown in a light-dark choice chamber. The results are totals of 5 trials.

Level of Maturation	Light	Dark	L/D ratio	L.S.*
Newly-emerged adults (June 6th)	94	6	15.66	0.005
Beginning of aestivation (July 5th)	10	90	0.11	0.005
Aestivation 75% complete (July 25th)	51	49	0.96	0.5 - 0.9
Aestivation complete (July 31st)	89	11	8.09	0.005

*level of significance from chi-square tests

odour of the females.

3.F.i. *Materials and methods*

The response of males to the odour of gravid females was evaluated by placing groups of 36 males in a darkened, moving-air olfactometer and employing 6 females as an odour source. The olfactometer (Fig. 3) is similar to that used by Hocking and Lindsay (1958) and consists of a Pyrex glass T-tube, 34.0 X 3.4 cm, including the end pieces. The outer surface was covered with black, vinyl tape to exclude light. The end pieces contained insect traps made from 20-mesh brass screen and were joined to the T-tube by means of polyvinyl chloride coupling sleeves which, according to the manufacturer, are odourless (Hocking and Lindsay, 1958). Laboratory service air was deodourized by passage through a 250 ml tower containing activated carbon, and moistened in a similar tower containing wet, 3mm glass beads. The air flow to the olfactometer was adjusted to 7 litres/minute (7.7 meters/minute) by means of a tapered-tube flow meter. The temperature of exhaust air from the system ranged between 19 and 21° C, while the relative humidity ranged between 70 and 80%. Test specimens were placed in the main body of the T-tube by means of the stem, and a strip of paper towelling served as a walking substrate for the beetles.

Before tests were conducted with females as an odour source, groups of 36 males were first subjected to 2 preliminary runs in moist air, one run with the air flow in one direction, the second run with the air flow reversed. These runs were of 1-hour duration each, and the numbers of beetles in upwind and

Table 4. The thigmokinetic response of aestivating adults of *Entomoscelis americana* Brown to openings in moist substrate in a moist-dry choice chamber. (Results are totals of 5 trials).

	End of chamber with no openings in substrate	End of chamber with openings in substrate:	
		In openings	Not in openings
Chamber with dry substrate (control)	50	9	41
Chamber with moist substrate openings	0	77	23

(Level of significance from chi-square test: 0.005)

down-wind traps were recorded at the conclusion of each run. Beetles that did not enter the insect traps were listed as neutral. Following the pairs of runs in moist air, 6 females were placed in the odour source tower and the pairs of runs repeated. The tower containing the females was washed and the system purged for 15 minutes with moist air between alternate runs to remove odour traces. A total of 10 pairs of runs was conducted with 5 groups of males: 5 pairs in moist air, and 5 pairs in moist air plus the odour of the females.

3.F.ii. *Results*

Table 5 shows the results of the tests in the moving air olfactometer. The results are the totals of the 10 pairs of runs listed in Appendix III. When moist air with no odour source was used, a total of 237 males remained in the main body of the olfactometer and were listed as neutral, while 62 entered the upwind trap and 61 entered the downwind trap. When gravid females were used as an odour source, a total of 133 remained in the main body of the olfactometer, while 198 entered the upwind trap and 29 entered the downwind trap.

Table 5. The response of males of *Entomoscelis americana* Brown to the odour of gravid females in a moving air olfactometer. (Results are totals of 5 trials).

	Neutral	Trap #1*	Trap #2
Moist air only	237	62	61
Moist air plus odour of females	133	198	29

(Level of significance from chi-square test: 0.005)

*odour source trap

4. FOOD PLANT RELATIONSHIPS OF E. AMERICANA

In oligophagous insects, successful utilisation of food plants depends largely upon properly co-ordinated interaction between the insect and the host plant (Dethier, 1954; Thorsteinson, 1960). While the behaviour that is involved in food plant selection has been divided into several components by a number of workers (Dethier, 1953; Thorsteinson, 1953; DeWilde, 1958), two major components were considered in this study:

(1) Orientation and movement toward the food plant, or food plant finding.

(2) Recognition of the food plant, or food plant acceptance.

4.A. Food plant finding by the larvae

The newly-hatched larvae need not search far for proper food plants since the adults deposit their eggs on soil beneath cruciferous plants. The proximity of hatching larvae to food plants gives rise to two distinct possibilities regarding food plant finding: (1) food plant finding is by trial and error and the larvae cannot recognize the proper plant until actual contact has been made or, (2) food plant finding is not random and the larvae will orientate and move toward the plant from a limited distance, by response to a stimulus such as odour. Experiments were conducted to evaluate the response of larvae to the odour of food plants, non-food plants, and to growing plants.

4.A.i. *Materials and methods*

Two groups of 10, newly-hatched larvae were placed in a pair of choice chambers in a darkened container. The choice chambers measured 23.0 X 12.0 cm, were covered with 20-mesh, brass screening, and were designed to fit over a pair of 10.0 cm diameter Pyrex glass petri dishes. In one choice chamber, fresh, whole rape leaves were placed in one petri dish and moistened paper towelling in the other. The second choice chamber acted as a control and fresh lettuce leaves were used in place of rape foliage. Previous tests revealed that the larvae will not eat lettuce. The two tests were run simultaneously and the location of the larvae in the choice chambers recorded 10 times at 15-minute intervals. The darkened container that held the choice chambers was rotated 90° at each check to discount the effects of light and laboratory air currents.

Five groups of 60, newly-hatched larvae were placed in the moving air olfactometer described in section 3.F.i. (Figure 3) for 1-hour periods. Each group of larvae was subjected to 2 pairs of runs in the olfactometer. The first pair of runs involved moist air only with the air flow direction reversed between runs, followed immediately by a pair of runs using freshly-picked, whole rape leaves as an odour source.

Groups of 100, newly-hatched larvae were each subjected to two runs in the olfactometer, each of 1-hour duration, to evaluate their response to the odour of non-food plants. Each group was first tested in moist air only, followed by a run using fresh, whole leaves of non-food plants as an odour source, and with the

air flow direction reversed. The non-food plants tested included leaf lettuce, *Lactuca sativa crispa* L., dandelion, *Taraxacum officinale* Weber, lamb's quarters, *Chenopodium album* L., and white Dutch runner beans, *Phaseolus coccineus albus* B.

One-hundred, newly-hatched larvae were offered a choice of growing food plants and non-food plants. Two plastic planters were used, one containing six, 6-inch rape plants, the other containing six, 6-inch lamb's quarters plants. The planters were set 10 cm apart in openings cut in the floor of a cardboard container that measured 60.0 X 30 cm at the floor and with walls 60 cm high. The container was open at the top and illuminated from above by a fluorescent light fixture equipped with four, 40-watt daylight type, 48-inch tubes. The incident light reaching the floor of the test chamber was measured with a photometer and the light source adjusted to provide even coverage. The larvae were placed on the floor of the test chamber at a point midway between the two planters. At 15-minute intervals, the locations of the larvae were recorded, and the test chamber rotated 90° to discount light direction and laboratory air currents. At each check, larvae climbing up the inner walls of the test chamber were returned to the floor, midway between the planters.

4.A.ii. *Results*

The tests with the 2-way, screened choice chambers did not indicate a positive larval response to the odour of rape foliage, and their distribution in the chambers was fairly even at the end

of the ten recordings (Appendix IV).

Table 6 shows the results of the tests in the moving air olfactometer and lists the totals of runs entered in Appendix V. In trials employing rape leaves as an odour source, significantly more larvae remained in the main body of the olfactometer than when moist air alone was used. When rape leaves were used, equal numbers of larvae were found in the insect traps at the conclusion of the runs, indicating that larval response to the odour of rape leaves is not orientated movement toward the odour source, but rather that larval movement is arrested in the presence of such odour. In the trials with lettuce, dandelion, and lamb's quarters, significant numbers of larvae moved away from the odour source when compared with runs in moist air, and were found in the down-wind insect traps. When bean foliage was used as an odour source, no significant difference was detected in larval movement when compared to runs in moist air.

Table 7 shows the numbers of larvae on food plants and non-food plants at 15-minute intervals during the tests with growing plants. Although approximately equal numbers of larvae climbed up both types of plants at the beginning of the test, those on rape plants soon settled down to feed, while the larvae on lamb's quarters plants continued climbing and walking on the leaves until most had fallen back to the soil. Very few larvae fell from the rape plants and the number of larvae on these plants increased at each 15-minute interval, while the number on the lamb's quarters plants correspondingly decreased.

Table 6. The response of first instar larvae of *Entomoscelis americana* Brown to the odours of food plants and non-food plants in a moving air olfactometer.

Type of Foliage	Moist air only (control)			Moist air plus plant odours			
	Neutral	Trap #1	Trap #2	Neutral	Trap #1*	Trap #2	L.S.**
Rape	125	236	239	190	205	205	0.005
Lettuce	102	220	178	72	48	380	0.005
Dandelion	128	210	162	96	72	332	0.005
Lamb's quarters	110	197	193	100	119	281	0.005
Bean	103	189	208	111	196	193	Not significant

*odour source

**level of significance from chi-square tests.

Table 7. The response of 100, newly-hatched larvae of *Entomoscelis americana* Brown to growing food and non-food plants in a choice chamber.

Time (mins)	Numbers on rape plants	Numbers on lamb's quarters plants	Numbers on walls and floor of choice chamber
15	18	20	62
30	26	24	50
45	28	21	51
60	37	18	45
75	36	16	48
90	41	12	47
105	42	14	44
120	49	9	42
135	56	12	32
150	58	10	32

4.B. Food plant finding by the adults

At the conclusion of the aestivation period, the beetles actively disperse and are then found on late-growth cruciferous plants that have an abundance of foliage and blossoms. Dispersing, field-collected adults were returned to the laboratory and tests conducted to determine their response to the odour of fresh rape foliage and blossoms.

4.B.i. *Materials and methods*

Groups of 20 adults, unfed for 24 hours, were placed in the moving air olfactometer described in section 3.F.i. (Figure 3) and tested in moist air only. Many of the test specimens entered the stem of the T-tube during the runs in moist air, and in order to overcome this difficulty the olfactometer was modified to provide two, separate air streams entering at the ends of the apparatus, and both exiting through the stem. This method was successful in that it provided air movement in the stem and very few specimens then entered the stem. A second 250 ml tower was added as an alternate odour source and the apparatus supplied with a second tapered tube, air flow-meter. The air flow entering each end was adjusted to 7 litres/minute (7.7 meters/minute). Five groups of 20 adults were each subjected to a pair of runs in the olfactometer, one run in moist air only, followed immediately by a run in which rape foliage or blossoms were employed as an odour source. Freshly-picked, whole leaves or blossoms were used. Runs were of 1-hour duration, and after each pair of runs, the odour source tower was

washed and the system purged with moist air for 15 minutes to remove odour traces. The odour source was placed in alternate towers in consecutive pairs of runs. In this fashion, 5 series of trials were conducted to evaluate the the olfactory response of:

(1) Males and females (mixed) to foliage;

(2) Females to foliage;

(3) Males to foliage;

(4) Females to blossoms;

(5) Males to blossoms.

4.B.ii. *Results*

Table 8 shows the results of the tests in the double air-stream olfactometer and lists the totals of 5 pairs of runs recorded in Appendix VI. When moist air alone was used, a large number of specimens remained in the main body of the olfactometer, while those that entered the insect traps displayed no distinct preference for either trap. When mixed males and females were exposed to the odour of rape foliage, 60% entered the odour trap. However, since these were mostly females, the sexes were separated in succeeding trials. When their response to the odour of rape foliage was checked separately, 75% of the females entered the odour trap, while the males showed no distinct preference. When the odour of rape blossoms was used, 87% of the females and 77% of the males were found in the odour trap.

Table 8. The response of adults of *Entomoscelis americana* Brown to odours of rape (*Brassica campestris* L.) foliage and blossoms in a double air-stream olfactometer.

	Moist air only (control)			Moist air plus plant odours			
	Neutral	Trap #1	Trap #2	Neutral	Trap #1	Trap #2	L.S.**
Foliage:							
Mixed adults	22	44	34	13	60	27	0.05
Females	38	27	35	14	75	11	0.005
Males	33	31	36	32	33	35	N.S.***
Blossoms:							
Females	63	20	17	9	87	4	0.005
Males	35	31	34	17	77	6	0.005

*odour source

**level of significance from chi-square tests

***not significant

4.C. Food plant acceptance

While phototaxis plays a major role in directing the larvae to the proper environment for feeding, and plant odours direct the adults to the proper plants, it is assumed that the ultimate forces that determine food plant acceptance, or final recognition, are largely gustatory response to chemicals in food plant tissue.

Although *E. americana* was not observed feeding on non-cruciferous plants in the field, tests were conducted to determine if the larvae or adults would feed on such plants. Other tests were conducted to determine if chemicals in rape seed foliage were responsible for food plant acceptance by *E. americana*, and whether both larvae and adults responded to the same chemicals. Finally, larval and adult responses to sinigrin, a mustard oil glucoside common to the Crucifereae, were tested.

4.C.i. *Materials and methods*

Bio-assay method - Figure 4 illustrates the test chamber used to evaluate the response of *E. americana* the non-cruciferous plants, to chemical fractions of rape foliage, and to sinigrin. Leaf discs of 2.0 cm diameter were prepared with a stainless steel punch and mounted on insect pins that were equipped with 0.5 cm diameter plastic discs to support the leaf discs. The insect pins were imbedded in a 1.0 cm layer of styrofoam plastic that was covered by a single layer of moist paper towelling and contained in a 10.0 cm diameter Pyrex glass petri dish. The leaf discs were held about 3 mm above the paper towelling during adult testing, and

about 1 mm for larval testing. The entire arrangement was covered with a stoppered, glass funnel.

Non-cruciferous plants - Groups of 10 adults, unfed for 24 hours, and groups of 50, newly-hatched larvae were offered non-cruciferous leaf discs in the test chambers for 4-hour periods. At the end of these periods, rape leaf discs were added to the test chambers, providing a choice of leaf discs for a second 4-hour period. Thirteen species of non-cruciferous plants, representing 5 families, were tested (Table 9).

Chemical fractions of rape foliage - Neither larvae nor adults of *E. americana*, starved for one day, will eat lettuce. In order to evaluate their response to chemical fractions of rape foliage, they were offered a choice of lettuce leaf discs treated with the chemical fractions, and untreated lettuce leaf discs. Choice trials involved groups of 10 adults, unfed for 24 hours, in the test chambers for 4-hour periods, and groups of 50, newly-hatched larvae for 12-hour periods. The adults were field-collected, and tested during the first 3 weeks after emergence. The larvae were from eggs hatched in the laboratory.

All chemical fractions from the plant extraction process were evaporated to dryness and dissolved in minimal amounts of methanol. Lettuce leaf discs were treated by immersion in the methanol solutions for one minute, and then air-dried. Control lettuce discs were immersed in methanol for one minute, and then air-dried. Immediately following each trial, the leaf discs were removed and their outlines accurately recorded on graph tracing

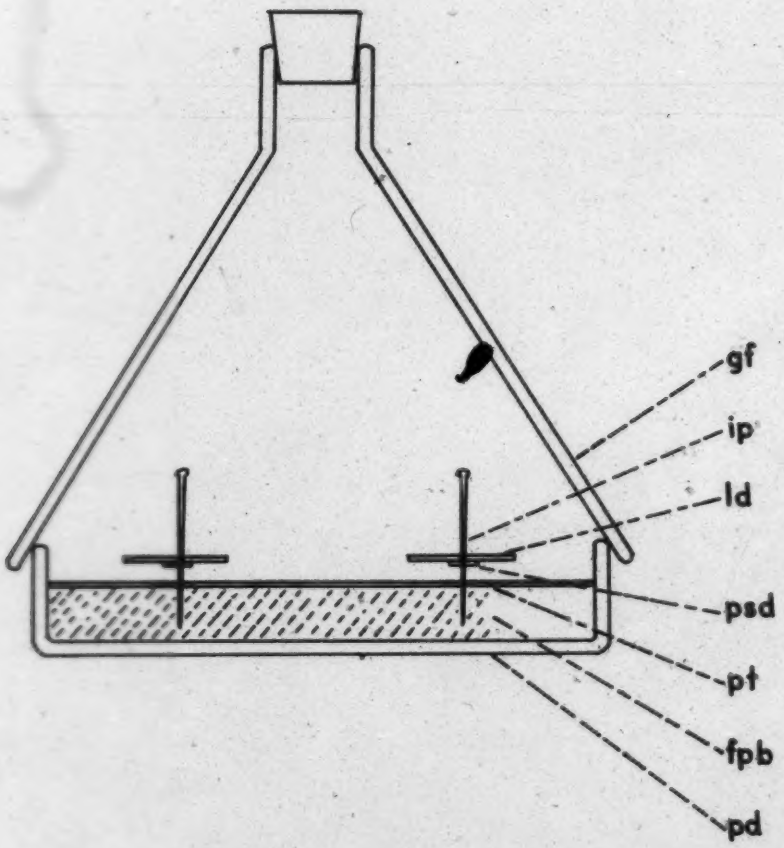

Fig. 4. The choice chamber used to evaluate the response of *Entomoscelis americana* Brown to food plants and non-food plants: gf, glass funnel; ip, insect pin; ld, leaf disc; psd, plastic supporting disc; pt, paper towelling; fpb, foam plastic bottomlayer; pd, petri dish.

Table 9. The non-cruciferous plants tested for feeding acceptability with *Entomoscelis americana* Brown.

Test plant	Common name
CHENOPODIACEAE	
Chenopodium album L.	lamb's quarters
Beta vulgaris L.	garden beet
Beta vulgaris cicla L.	Swiss chard
Spinacia oleracea L.	spinach
LEGUMINOSEAE	
Pisum sativum L.	garden pea
Medicago sativa L.	alfalfa
TROPAEOLACEAE	
Tropaeolum majus L.	large nasturtium
SOLANACEAE	
Solanum tuberosum L.	potato
Lycopersicon esculentum Mill.	tomato
Nicotiana tabacum L.	tobacco
COMPOSITAE	
Taraxacum officinale Weber	dandelion
Cirsium arvense (L.) Scop.	Canada thistle
Lactuca sativa crispa L.	leaf lettuce

paper. These were then reproduced by direct contact on Kodalith photographic film (Canadian Kodak Co., Toronto, Ont.), and enlarged 5.66 times with a projector. The enlarged image provided a circle with an area of 100 cm^2 which, when traced with a polar planimeter (Hughes and Owens Co., Toronto, Ont.) gave a reading of 100 when the instrument was set to yield a 1:1 ratio. The ratios of the areas of the partially-consumed discs to their original areas were determined, and the areas consumed expressed as percentages of the whole.

Two methods of chemical fractionation of rape foliage were carried out in this study. The first method involved the fractionation of solvent extracts by means of ion-exchange chromatography. This system was abandoned since it failed to concentrate the chemical feeding stimulants in definite fractions. The second fractionation method was designed, with minor modifications, after the method used by Nayar and Thorsteinson (1963) to isolate mustard oil glucosides from plant tissues of species in the families Crucifereae and Tropaeolaceae. Fig. 5 is a flow diagram of the fractionation procedure employed in this study.

Fresh rape leaves were dried in an electric, ventilated oven (Cenco Instruments Corp., Toronto, Ont.) set at 80° C, this temperature selected to inactivate naturally-occurring plant enzymes that may tend to break down compounds. (As noted in section 3.D.i., rape leaves detached for 3 days are toxic to the larvae.) The dried leaves were ground to a powder in a Sorval Omni-Mixer and stored in glass jars at 0° C. A 100-gram sample of the leaf powder was extracted 3 times in petroleum ether with a Sorval Omni-Mixer.

Each extraction was for 30 minutes in 300 ml of petroleum ether that was removed by filtration between extractions. The temperature during the extraction procedure was held near 20° C by means of an ice-water bath. The liquid fractions were combined, reduced by evaporation, and tested for a feeding stimulant with *E. americana*. Larvae and adults were each subjected to 5 trials in test chambers to evaluate their response to this fraction.

The residue from the petroleum ether extraction process was then extracted 3 times in 80% ethanol, following the same procedure employed in the petroleum ether extraction, and was similarly tested with *E. americana*. Since the ethanol solution contained a feeding stimulant, it was reduced to 10 ml by evaporation, and chromatographed on a 2.0 X 36.0 cm column of Adsorption Alumina, 80-200 mesh (Fisher Scientific Co., Fair Lawn, N.J.), packed and eluted with 80% ethanol. Column flow was adjusted to 30 ml/hour and 10-ml fractions collected with an automatic rotary fraction collector (Buchler Instruments Corp., Fort Lee, N.J.). Each 10-ml fraction was tested for a feeding stimulant with the larvae and adults of *E. americana*. Since a feeding stimulant was present in fractions 6-25, these were combined, reduced to 10 ml by evaporation, and chromatographed on a 2.0 X 36.0 column packed with a cellulose slurry in, and eluted with, 70% ethanol. 10-ml fractions were again collected and, when tested with *E. americana*, a feeding stimulant was detected in fractions 5-16. These fractions were combined, reduced to dryness by evaporation, dissolved in 20 ml of distilled water, and placed on a 2.0 X 36.0 column of activated

carbon (J. T. Baker Chemical Co., Phillipsburg, N.J.). This column was eluted with 250 ml of distilled water, followed by like amounts of ethanol solutions in concentrations 30, 60, and 90%, respectively. Each of the 4 resulting fractions was tested 5 times with the larvae and adults of *E. americana*. Since a feeding stimulant was present in the water and 60% eluates, these were combined, evaporated to dryness, and dissolved in 100 ml of distilled water at 50° C. The water solution was cooled to 21° C and shaken for 10 minutes in a separatory funnel with 100 ml of diethyl ether. The resulting 2 fractions were each tested 5 times for a feeding stimulant with the larvae and adults. Finally, the 2 fractions were re-combined and again tested with *E. americana*.

Tests with sinigrin - A 0.1 gm sample of sinigrin (Nutritional Biochemical Corp., Cleveland, Ohio) was dissolved in 10 ml of distilled water at 21° C. The larvae and adults were each tested 5 times in choice chambers, employing lettuce leaf discs that had been immersed in the sinigrin solution for one minute and air-dried. Control discs were immersed in distilled water for one minute and air-dried.

4.C.ii. *Results*

Non-cruciferous plants - Of the 13 species of non-cruciferous plants tested, none were accepted by *E. americana*. In all tests, when fresh rape leaf discs were added during the second 4-hour period, these were readily accepted by both larvae and adults.

Chemical fractions of rape foliage - Table 10 lists the results of the tests with chemical fractions of rape foliage. The control leaf discs remained intact during all tests and are not included in the table. *E. americana* did not accept leaf discs treated with the petroleum ether fraction; however, both larvae and adults accepted leaf discs treated with the 80% ethanol extract and each consumed about 49.8% of the leaf areas. The results of individual trials with the ethanol extract fraction are listed in Appendix VII.

Of the fractions collected from the adsorption alumina column, the larvae accepted fractions 11-17, the adults, 6-25. Greatest acceptance by both larvae and adults was detected in fractions 13-15. The larvae accepted fractions 7-14 from the cellulose slurry column, the adults, 5-16. Greatest response was to fraction 9, for leaf discs treated with this fraction were entirely consumed by both larvae and adults. *E. americana* did not accept leaf discs treated with the 90 and 30% eluates from the activated carbon column. Of the leaf discs treated with the distilled water and 60% ethanol eluates, the larvae consumed an average of 72.6 and 30.6%, respectively, the adults, 34.8 and 30.8%, respectively. These averages are computed from a total of 5 trials each for the larvae and adults, listed in Appendix VII.

Both larvae and adults accepted leaf discs treated with the water layer from the separatory funnel, although larval response was greater. As the result of 5 trials each (Appendix VII),

Table 10. The response of *Entomoscelis americana* Brown to lettuce leaf discs treated with chemical fractions of rape (*Brassica campestris* L.) foliage.

Fraction	Approximate area of discs consumed (%)	
	Larvae	Adults
Petroleum ether extract (Result of 5 trials)	0	0
80% ethanol extract (Average of 5 trials)	49.8	49.8
Adsorption alumina column		
Fraction No:		
5	0	0
6	0	6
7	0	13
8	0	19
9	0	19
10	0	25
11	8	38
12	13	36
13	18	29
14	61	84
15	32	61
16	23	47

Table 10 (continued)

Fraction	Approximate area of discs consumed (%)	
	Larvae	Adults
Adsorption alumina column		
Fraction No:		
17	11	35
18	0	42
19	0	31
20	0	22
21	0	19
22	0	16
23	0	16
24	0	13
25	0	12
26	0	0
Cellulose slurry column		
Fraction No:		
4	0	0
5	0	26
6	0	28
7	20	36
8	32	30
9	100	100
10	92	100

Table 10 (continued)

Fraction	Approximate area of discs consumed (%)	
	Larvae	Adults
Cellulose slurry column		
Fraction No:		
11	63	72
12	46	63
13	49	52
14	40	23
15	0	24
16	0	20
17	0	0
Activated carbon column		
(Average of 5 trials)		
90% ethanol	0	0
60% ethanol	30.6	30.8
30% ethanol	0	0
Distilled water	72.6	34.8
Separatory funnel fractions		
(Average of 5 trials)		
Water layer (1)	45.4	21.6
Diethyl ether layer (2)	0	6.2
(1) & (2) combined	48.8	73.4

the larvae consumed an average of 45.4% of the treated leaf discs, the adults about 21.6%. The larvae did not accept leaf discs treated with the diethyl ether layer, while the adults displayed a slight response, consuming an average of 6.2% of the leaf areas. When the 2 fractions from the separatory funnel were re-combined, no significant change was detected in larval response and they consumed an average of 48.8% of the leaf area. Such re-combination did significantly affect adult response however, for they consumed an average of 73.4% of the treated leaf area.

Tests with sinigrin - *E. americana* did not accept leaf discs treated with sinigrin.

4.D. Preliminary tests to classify plant compounds

Preliminary tests were conducted to classify the unknown compounds in the water and diethyl ether layers from the separatory funnel. The scope of the tests was limited due to the small amount of residue obtained, approximately 0.1 g from the diethyl ether layer and about 0.01 g from the water layer.

Diethyl ether layer - Evaporation of the diethyl ether layer yielded a dark-brown, viscous substance that would not dry completely when exposed to air, and had a distinctive, fruity odour. This substance is insoluble in water at room temperature, and soluble in dilute sodium hydroxide and dilute sodium bicarbonate. According to Shriner, Fuson, and Curtin (1967), low volatility combined with these solubility properties indicates either a high-molecular-weight acid or a substituted phenol. The distinctive odour suggests

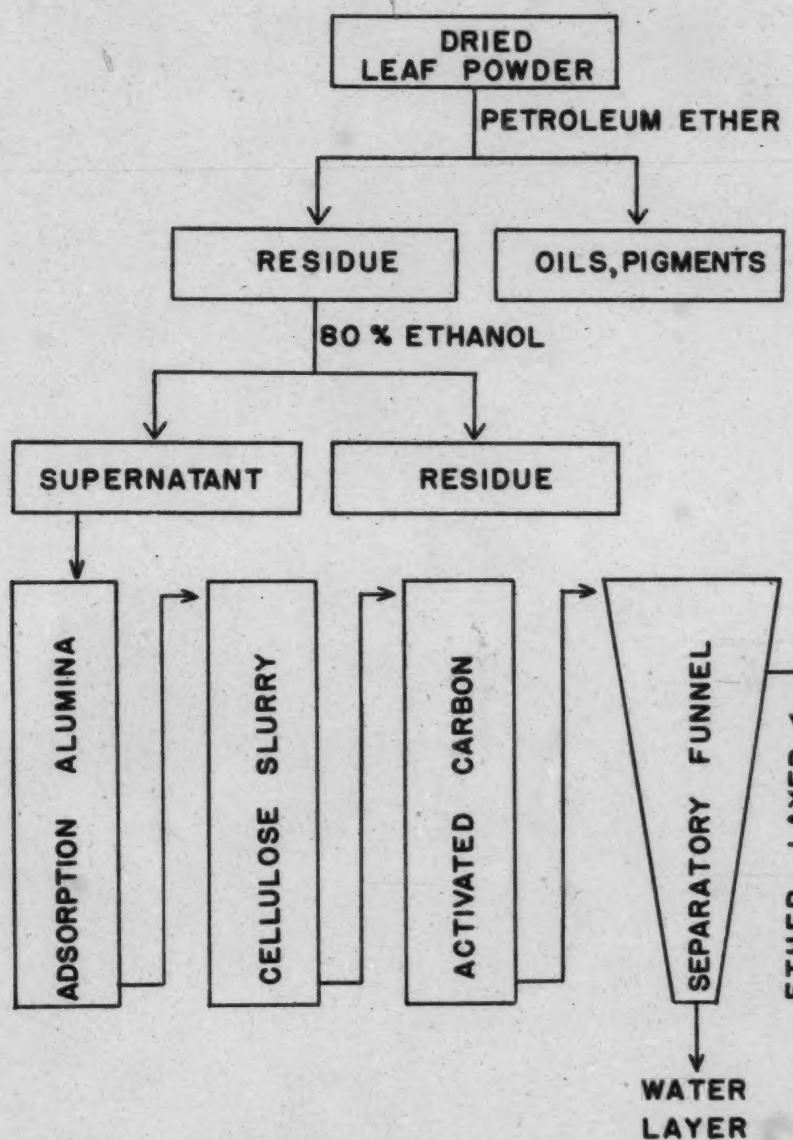

Fig. 5. A flow diagram of the procedure used to refine a chemical feeding stimulant from rape (*Brassica campestris* L.) foliage.

the latter.

Water layer - The water layer fraction has a pH of 6.9, is odourless, and yields a positive reaction with Benedict's solution. According to Shriner, Fuson, and Curtin (1967), a positive reaction to Benedict's solution combined with no solubility in diethyl ether indicates the presence of an aldehyde with a free reducing group. Control tests, using Benedict's solution, were run with glucose and sinigrin, and both yielded a positive reaction.

CONCLUSION AND DISCUSSION

The distribution of *E. americana* in North America and the habits of the stages indicate adaptation to a cool, fairly dry, continental type climate. Winters are spent in the soil as eggs, diapausing in the late embryo stage, while the warmest summer month in Alberta is spent in aestivation.

E. americana is oligophagous and restricted to the Cruciferae. Hatching occurs near cruciferous plants at a time when these plants are in the seedling stage, and the larvae need not search far for food. Phototaxis plays a major role in directing the larvae to the proper environment for feeding and this may be assisted by geotaxis and hygrotaxis. Although no directed movement by larvae toward food plant odours was demonstrated, such odours act as arrestants at very close range, possibly when the larvae are walking on the food plant, while non-food plant odours can act as repellents. That these two effects may combine to direct the larvae to the proper plant for feeding was demonstrated by the tests with growing food and non-food plants (Table 7). Thorsteinson (1960) contended that food plant finding by phytophagous insects is largely by chance, and no response to food plant odours was reported by Pospicil (1972) in larvae of several lepidopteran and coleopteran pests of sugar cane. Although Chin (1950) reported that Colorado potato beetle larvae responded to the odours of potato foliage from a distance of about 5mm, Bongers (1970) concluded that such re-

sponse is not necessary for survival since the eggs are normally deposited on potato leaves. Similarly, the eggs of *E. americana* are laid on soil beneath food plants and long range response to food plant odours by the larvae is not critical for survival.

Although no response to food plant odours was detected in newly-emerged adults, food plant finding is not critical at this time since the plants that served as larval food still have an ample supply of foliage. During *E. americana*'s July aestivation period, these plants form seed pods and the leaves senesce. Consequently, dispersal and food plant finding is critical after aestivation, and the response to food plant odours coincides with active dispersal in early August.

The striking reversals of phototaxis displayed by the adults coincides with other behavioural changes. Newly-emerged adults are strongly photo-positive (Table 3) and are found on plant stems. The reversals of phototaxis at the beginning and end of July are in synchrony with the aestivation period. The photo-negative response of aestivating adults is coupled with a positive response to moisture and a thigmokinetic response in conjunction with moist substrate. These 3 responses combine to direct *E. americana* to the proper environment for aestivation, since this period is spent under leaf litter and in soil crevices. The second reversal of phototaxis coincides with the termination of the aestivation period.

Copulation was not observed until immediately after dispersal in early August, at which time males responded to female

odours. Copulation after dispersal prevents inbreeding in a local population and increases genetic variation through exchange with other populations.

During the rape foliage fractionation process and later tests to characterize chemical feeding stimulants, it was demonstrated that an aldehyde stimulates larval feeding and synergises with a compound that has phenolic properties to stimulate adult feeding. The fact that larval response is confined to the aldehyde indicates that it is not a nutrient phagostimulant such as a sugar, but more likely an aldehyde peculiar to the Crucifereae, since the larvae are restricted to this plant family. Although the larvae do not respond to sinigrin or to nasturtium foliage which contains sinigrin, a mustard oil glucoside cannot be ruled out as a feeding stimulant since several such glucosides may occur in a single plant (Nayar and Thorsteinson, 1963). Adult feeding response was greatest to the combined effects of the aldehyde and a compound with phenolic properties. Hsiao and Fraenkel (1961) reported that a phenolic compound in potato foliage may be a specific stimulant for the Colorado potato beetle, and as discussed in the literature review, cases of synergism in phytophagous insects has been widely reported.

Since little previous research regarding *E. americana* has been reported, this study can form the basis for further investigations into the biology, food plant relationships, and control of *E. americana*. The description of the stages will aid in field identification, while the information regarding rearing techniques and termination of diapause will be useful in establishing laboratory

colonies. The information regarding the habits of the stages and food plant finding is useful in developing cultural control methods. *E. americana* has recently attracted attention as an economic pest because of increasing rape acreage in Western Canada, and will continue to be a pest as long as volunteer rape is allowed to spread through agricultural lands. Crop rotation, spring tillage, and more efficient methods of rape seed harvesting will help to suppress volunteer rape and can be investigated as methods of cultural control of *E. americana*.

Further investigations can be conducted to identify the plant compounds in rape foliage that initiate feeding by *E. americana*. These chemicals may then be used as bait in insect traps to estimate field population densities. Perhaps varieties of rape lacking these chemical can be developed, much in the same way that rape varieties with low erucic acid content were developed (Williams, 1972).

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7. APPENDICES

Appendix I. The photo-response of adults of *Entomoscelis americana* Brown in a light-dark choice chamber.

Level of maturation	Run	Light	Dark
Newly-emerged adults	1	19	1
	2	20	0
	3	20	0
	4	18	2
	5	17	3
Beginning of aestivation	1	3	17
	2	2	18
	3	0	20
	4	1	19
	5	4	16
Aestivation 75% complete	1	8	12
	2	13	7
	3	11	9
	4	8	12
	5	9	11

Appendix I (continued)

Level of maturation	Run	Light	Dark
Aestivation complete	1	18	2
	2	19	1
	3	17	3
	4	16	4
	5	19	1

Appendix II. The thigmokinetic response of aestivating adults of *Entomoscelis americana* to openings in a moist substrate in a moist-dry choice chamber.

	Run	End of chamber with no openings in substrate	End of chamber with openings in substrate:	
			In Openings	Not in openings
Chamber with no moist substrate (control)	1	12	2	6
	2	10	1	9
	3	6	2	12
	4	8	4	8
	5	14	0	6
Chamber with moist substrate openings	1	0	16	4
	2	0	17	3
	3	0	14	6
	4	0	16	4
	5	0	14	6

Appendix III. The response of males of *Entomoscelis americana* Brown to the odour of gravid females in a moving air olfactometer.

	Moist air only (control)				Moist air plus odour of females		
Run	Neutral	Trap #1	Trap #2	Run	Neutral	Trap #1*	Trap #2
1a	12	12	12	1c	4	26	6
1b	16	10	10	1d	8	24	4
2a	32	0	4	2c	20	12	4
2b	28	4	4	2d	16	16	4
3a	30	2	4	3c	19	19	0
3b	27	6	3	3d	15	17	4
4a	22	8	6	4c	15	21	0
4b	22	10	4	4d	14	20	2
5a	24	6	6	5c	12	24	0
5b	24	4	8	5d	12	19	5

*odour source

Appendix IV. The response of first instar larvae of *Entomoscelis americana* to the odour of rape foliage in 2-way, screened choice chambers.

Time of check (mins.)	Choice chamber with lettuce		Choice chamber with rape	
	Odour source	No odour source	Odour source	No odour source
15	7	3	7	3
30	5	5	5	5
45	6	4	4	6
60	7	3	6	4
75	4	6	7	3
90	5	5	2	8
105	4	6	3	7
120	3	7	2	8
135	4	6	6	4
150	5	5	5	5

Appendix V. The response of first instar larvae of *Entomoscelis americana* Brown to the odours of food plants and non-food plants in a moving air olfactometer.

Type of foliage	Moist air only (control)				Moist air plus plant odours			
	Run	Neutral	Trap #1	Trap #2	Run	Neutral	Trap #1*	Trap #2
Rape	1a	12	20	28	1c	14	32	14
	1b	12	22	26	1d	16	18	26
	2a	13	25	22	2c	15	28	17
	2b	13	27	20	2d	15	24	21
	3a	12	23	25	3c	16	24	20
	3b	14	28	18	3d	14	16	30
	4a	12	20	28	4c	20	17	23
	4b	17	17	26	4d	36	12	12
	5a	10	24	26	5c	20	18	22
	5b	10	20	30	5d	24	16	20

*odour source

Appendix V. (continued)

Type of foliage	Moist air only (control)				Moist air plus plant odours			
	Run	Neutral	Trap #1	Trap #2	Run	Neutral	Trap #1*	Trap #2
Lettuce	1a	20	42	38	1b	11	4	85
	2a	28	40	32	2b	14	7	79
	3a	16	48	36	3b	17	13	70
	4a	24	43	33	4b	18	17	65
	5a	14	47	39	5b	12	7	81
Dandelion	1a	28	39	33	1b	12	10	78
	2a	24	44	32	2b	22	14	64
	3a	30	36	34	3b	26	18	56
	4a	22	50	28	4b	20	19	61
	5a	24	41	35	5b	16	11	73

*odour source

Appendix V. (continued)

Type of foliage	Moist air only (control)				Moist air plus plant odours		
	Run	Neutral	Trap #1	Trap #2	Run	Neutral	Trap #1*	Trap #2
Lamb's quarters	1a	20	38	42	1b	22	4	74
	2a	32	36	32	2b	18	16	66
	3a	18	49	33	3b	17	10	73
	4a	14	40	46	4b	12	9	79
	5a	26	34	40	5b	21	2	77
Bean	1a	18	42	40	1b	20	32	48
	2a	24	40	36	2b	30	36	34
	3a	16	32	52	3b	20	41	39
	4a	30	32	38	4b	22	43	35
	5a	15	43	42	5b	19	44	37

*odour source

Appendix VI. The response of adults of *Entomoscelis americana* to odours of rape foliage and blossoms in a double air stream olfactometer.

	Control (no odour source)				Experimental (odour source)			
	Run	Neutral	Trap #1	Trap #2	Run	Neutral	Trap #1	Trap #2
Foliage:								
Mixed adults	1	6	8	6	1a	2	8*	6
	2	5	10	5	2a	5	6	9*
	3	4	9	7	3a	2	12*	6
	4	4	8	8	4a	2	4	14*
	5	3	9	8	5a	2	13*	5
Females	1	12	2	6	1a	6	14*	0
	2	10	6	4	2a	2	2	16*
	3	5	8	7	3a	1	18*	1
	4	5	14	1	4a	2	6	12*
	5	6	4	10	5a	3	15*	2

*odour source

Appendix VI. (continued)

		Control (no odour source)			Experimental (odour source)			
	Run	Neutral	Trap #1	Trap #2	Run	Neutral	Trap #1	Trap #2
Foliage:								
Males	1	18	10	12	1a	20	10*	10
	2	16	14	10	2a	10	14	16*
	3	8	12	20	3a	10	15*	15
	4	13	17	10	4a	12	16	12*
	5	11	9	20	5a	12	14*	14
Blossoms:								
Females	1	13	4	3	1a	2	18*	0
	2	13	5	2	2a	0	1	19*
	3	12	2	6	3a	2	17*	1
	4	9	6	5	4a	4	1	15*
	5	16	3	1	5a	1	18*	1
Males	1	8	6	6	1a	2	16*	2
	2	8	4	8	2a	8	0	12*
	3	9	7	4	3a	3	15*	2
	4	5	5	10	4a	4	0	16*
	5	5	9	6	5a	0	18*	2

*odour source

Appendix VII. The response of *Entomoscelis americana* to lettuce leaf discs treated with chemical fractions of rape seed foliage.

	Trial	Approximate area of discs consumed (%) Larvae	Adults
80% ethanol extract:	1	55	49
	2	60	59
	3	44	68
	4	43	22
	5	47	51
Activated carbon column:			
60% ethanol eluate	1	39	23
	2	33	28
	3	32	26
	4	30	32
	5	19	45
Distilled water eluate	1	75	43
	2	79	36
	3	63	37
	4	72	28
	5	74	30

Appendix VII. (continued)

	Trial	Approximate area of discs consumed (%) Larvae	Adults
Separatory funnel fractions:			
Water layer (1)	1	47	18
	2	44	22
	3	46	25
	4	53	26
	5	47	17
Ether layer (2)	1	0	5
	2	0	7
	3	0	4
	4	0	6
	5	0	9
(1) & (2) combined	1	65	77
	2	50	79
	3	45	76
	4	38	64
	5	46	71

AUTOBIOGRAPHICAL SKETCH

I was born in Bowsman, Manitoba in 1927 and received my primary education at small, rural schools in central British Columbia. Following completion of Grade IX, I left school in 1942 - not because of a lack of interest, but for economic reasons. I then spent 4 years with various forest product companies in British Columbia and, since these years were spent in forested areas, I developed a keen interest in biological diversity and relationships. I then served over 6 years with the R.C.M. Police, chiefly in rural detachments in the Peace River district of Alberta. I spent the next 14 years in Edmonton where I was employed in the personnel department of Celanese Canada Ltd.

I picked up the threads of my formal education in 1965 by registering for evening high school courses offered by the Edmonton Public School Board and received a Senior Matriculation in 1967. Since I encountered little difficulty with the high school program, I decided to continue my education on a full-time basis and enrolled in Simon Fraser University in January, 1968.

A strong penchant for the life sciences led to my choice of biology at Simon Fraser University, and because of their tri-semester system, I was able to complete a 4-year program in 3 years. I was awarded a B.Sc. (Hons.) in 1971. While at Simon Fraser University, I became profoundly interested in the unusual world of insects and decided to study entomology at the graduate level.

During botany field trips in British Columbia, I was introduced to some of the many specificities existing between plants and insects. A keen interest in these relationships led to the undertaking of the research described in this thesis.